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Research Article

Morphological characteristics and phylogenetic analyses revealed four new species (Basidiomycota) in the Yunnan-Guizhou Plateau, China

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Abstract

Four new fungi, *viz. Clavulinopsis wumengshanensis* (Clavariaceae, Agaricales), *Henningsomyces bambusae* (Porotheleaceae, Agaricales), *Xenasma bisterigmatae*, and *X. guttulata* (Xenasmataceae, Russulales), from Yunnan Province in China, are proposed, based on a combination of morphological features and molecular evidence. Phylogenetic analyses were conducted using a combined dataset of internal transcribed space and nuclear ribosomal RNA large subunit sequences. The ITS+LSU analysis showed that *Clavulinopsis wumengshanensis* **sp. nov.** groups with *C. aurantiocinnabarina*, *Henningsomyces bambusae* **sp. nov.** forms a sister group with *H. candidus*, *Xenasma bisterigmatae* **sp. nov.** is clustered with *X. rimicola*, and *X. guttulata* **sp. nov.** is clustered with *X. pruinosum*. The morphology and multi-gene phylogenetic analyses confirmed the placement of the four new taxa. *Clavulinopsis wumengshanensis* is distinguished by buff-yellow to straw-yellow basidiomata, clavate to subcylindrical basidia with four sterigmata, and subglobose basidiospores with several guttules (7–8.5 × 6–7.5 µm); *Henningsomyces bambusae* is characterized by white to cream basidiomata with short cylindric to tubular colonies, cylindrical to subcylindrical basidia with two sterigmata and globose to subglobose basidiospores (6.5–8.5 × 6.5–8.5 µm); *Xenasma bisterigmatae* can be characterized by its membranaceous and ash-grey basidiomata, clavate to subcylindrical basidia with two sterigmata and ellipsoid to subglobose basidiospores (10–12.5 × 8–10.5 µm); *X. guttulata* is distinguished by membranaceous and white to cream basidiomata, clavate to subcylindrical basidia with two sterigmata and ellipsoid to narrowly ellipsoid basidiospores (7–9 × 5.5–7.5 µm).

Key words: 4 new taxa, Agaricales, biodiversity, Russulales, taxonomy; Yunnan province

Introduction

The phylum Basidiomycota R.T. Moore represents one of the major divisions in the fungal tree of life, with global estimates that it encompasses 1.4–4.2 million species in the phylum and the latest estimates of 0.7 to 1 million species, which

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represent about 28–40% of all fungal diversity (Hyde 2022; He et al. 2024; Liu et al. 2024). The basidiomata of Basidiomycota exhibit complex forms, such as coralloid, corticioid, gilled, hydnoid, poroid, and toothed basidiomata (Bernicchia and Gorjón 2010; He et al. 2019; Zhou et al. 2024a). Traditionally, Basidiomycota is typically characterized by the basidia and basidiospores and some variable morphological characters, such as diverse cellular constructions in hyphal systems and meiosporangia; the basidiomata reflect a profound evolutionary history at the various taxonomic levels within Basidiomycota (Zhao et al. 2016; Hyde et al. 2023; Dong et al. 2024). Nowadays, DNA sequence-based classification and identification have become the standard methodology in fungal taxonomy (Dai et al. 2021; Zhang et al. 2023; Dong et al. 2024; He et al. 2024).

The genus *Clavulinopsis* Overeem, classified within the family Clavariaceae (Agaricales, Basidiomycota), includes species that are widely distributed. It is characterized by yellow, orange, or creamy white basidiomata, with simple or regularly dichotomously branched, cylindric or fusoid stems; generative hyphae with obtuse, occasionally inflated hyphae with clamp connections; 2–4-spored basidia; and smooth or echinulate basidiospores occasionally with big guttules (Petersen 1996; Knudsen and Vesterholt 2012; Keleş and Kaya 2021; Yan et al. 2023). More than 171 taxon records of the genus currently are listed in the Index Fungorum database (http://www.indexfungorum.org; accessed on 2 November 2024), and approximately 84 species names are legitimately published (Yan et al. 2023). However, the phylogeny of *Clavulinopsis* is ambiguous due to a lack of molecular evidence and morphological data. Two similar genera, *Clavaria* and *Ramariopsis*, are easily confused with *Clavulinopsis* in clavarioid basidiomata. However, the micromorphology feathers are different between the three genera; *viz.*, *Ramariopsis* has various basidiomata, but those in *Clavaria* and *Clavulinopsis* are simple; clamp connections are present on the basidia and hyphae in *Clavulinopsis* (Corner 1950, 1970; Petersen and Corner 1968; Yan et al. 2023). Furthermore, the outline of *Clavulinopsis* was defined by the basidiospore ornamentation, and the related taxa were examined by D.N. Pegler and T.W.K. Young. Applying the molecular phylogenetic methods generated considerable promotion in the generic definition of *Clavulinopsis*, gradually clarifying the classification boundaries among several closely related genera, particularly *Clavaria* and *Ramariopsis* (Furtado et al. 2016; Yan et al. 2023; He et al. 2024).

Henningsomyces Kuntze, a type of cyphelloid fungi first described by Kuntze (1898), belongs to the family Porotheleaceae (Agaricales) (He et al. 2024). Previously, cyphelloid fungi were classified in the family Cyphellaceae based on similar morphology. Subsequently, the name Porotheleaceae (Murrill 1916) was later established for this group (Cooke 1957, 1961). However, the phylogenetic relationship of many cyphelloid fungi remains ambiguous. The genus *Henningsomyces*, a typical cyphelloid fungus with cylindric basidiomata, is typified by *H. candidus* (Pers.) Kuntze and is characterized by annual basidiomata consisting of sparse or gregarious tubes, a monomitic hyphae system that typically exhibits both clamp connections and simple septa, an absence of cystidia, and globose to subglobose basidiospores (Wei and Qin 2009; Liu et al. 2023). Among the 50 records of the genus currently listed in the Index Fungorum database (http://www.indexfungorum.org; accessed on 2 November 2024), approximately 22 species names are legitimately published (Liu et al. 2023). Among those studies, *Henningsomyces* forms a monophyletic lineage and nests into

the order Agaricales (Moncalvo et al. 2006, Bodensteiner et al. 2004, Binder et al. 2005, Thorn et al. 2005, Baltazar et al. 2015, Lucas and Dentinger 2015, Moreno et al. 2017). However, the placement of *Henningsomyces* and related taxa has not yet been substantiated. In recent years, based on morphological examination and molecular phylogenetic analysis, six new species were described in China (Yan et al. 2023). Most mycologists focused on the poroid or corticioid species, but the cyphelloid species bearing cup-, bowl-, or tube-shaped "cyphelloid" hymenophores were rarely reported, such as the genus *Henningsomyces* bearing the cylindric basidiomata (Wei and Qin 2009).

Xenasma Donk, classified in the family Xenasmataceae (Russulales, Basidiomycota), was introduced in 1957 and is typified by *X. rimicola* (P. Karst.) Donk (Liberta 1960; Bernicchia and Gorjón 2010). This genus is characterized by the resupinate and smooth basidiomata, a monomitic hyphal system with clamps, generative hyphae, and globose to cylindrical, striate basidiospores (Liberta 1960; Bernicchia and Gorjón 2010). Among the 39 records of the genus currently listed in the Index Fungorum database (http://www.indexfungorum.org; accessed on 2 November 2024), only 11 species names are legitimately published (Bernicchia and Gorjón 2010; He et al. 2024). *Xenasma* Donk continues to intrigue mycologists due to its unique morphological characteristics and ecological roles (Bernicchia and Gorjón 2010). The genus is primarily found in temperate forest ecosystems, often growing on decaying wood and contributing to wood decomposition (Liu et al. 2024). Recent phylogenetic studies have expanded the understanding of its evolutionary relationships within the Russulales, highlighting the potential for undiscovered species in understudied regions (He et al. 2019, 2024). The morphology of the basidiome and hymenophore, together with habitat, are often regarded as important characters for the taxonomy of the order Russulales, and Xenasmataceae is the only family in which the smooth hymenophore configuration could be found (Larsson and Larsson 2003; Miller et al. 2006; He et al. 2024). In the latest study, there are only two genera in the family Xenasmataceae, namely *Xenasma* and *Xenosperma* Oberw, in which the genus *Xenasma* is a mystery genus, and no new taxon was reported from this genus for nearly half a century (He et al. 2024).

The specimens of the three genera collected in the Yunnan-Guizhou Plateau, China, which could not be assigned to any described species of the order. Therefore, four new species, *viz. Clavulinopsis wumengshanensis*, *Henningsomyces bambusae*, *Xenasma bisterigmatae*, and *X. guttulate*, are proposed with descriptions, illustrations, and phylogenetic analysis results.

Materials and methods

Morphology

Fresh fruiting bodies of the fungi were collected from Wumengshan National Nature Reserve in Zhaotong of Yunnan Province, China, and the important collection information was recorded (Rathnayaka et al. 2024). Specimens were dried in an electric food dehydrator at 40 °C (Hu et al. 2022), then sealed and stored in an envelope bag and deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, China. Macromorphological descriptions are based on field notes and photos captured in the field and lab. Color terminology follows Petersen (1996). Micromorphological data were

obtained from the dried specimens when observed under a light microscope following the previous study (Zhao et al. 2023; Zhou et al. 2024b). The following abbreviations are used: KOH = 5% potassium hydroxide water solution, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's Reagent, IKI– = both inamyloid and indextrinoid, Lm = mean spore length (arithmetic average for all spores), Wm = mean spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied, and n = a/b (number of spores (a) measured from given number (b) of specimens).

Molecular phylogeny

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing) was used to obtain DNA from dried specimens, and PCR was performed according to the manufacturer's instructions with some modifications. ITS locus was amplified using the primer pairs ITS5/ITS4 (White et al. 1990). The nuclear LSU region was amplified with primer pair LR0R and LR7 (Vilgalys and Hester 1990). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 54 °C for 45 s, and 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR procedure for LSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 1 min, and 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. All newly generated sequences were submitted to GenBank and are listed in Table 1.

Sequences generated for this study were aligned, with additional sequences downloaded from GenBank. Sequences were aligned using MAFFT v.7 (https://mafft.cbrc.jp/alignment/server/), adjusting the direction of nucleotide sequences according to the first sequence (accurate enough for most cases) and selecting the G-INS-i iterative refinement method (Katoh et al. 2019). Alignments were manually adjusted to maximize alignment and minimize gaps with BioEdit v.7.0.9 (Hall 1999). A dataset of concatenated ITS and LSU sequences was used to determine the phylogenetic position of the new species. Maximum likelihood (ML) analysis was performed using the CIPRES Science Gateway (Miller et al. 2010) based on the dataset using the RA × ML-HPC BlackBox tool, with the setting RA × ML halt bootstrapping automatically and 0.25 for maximum hours and obtaining the best tree using ML search. Other parameters in ML analysis followed default settings, and statistical support values were obtained using nonparametric bootstrapping with 1,000 replicates. Maximum parsimony (MP) analyses were applied to the combined three datasets following the methods outlined in a previous study (Zhao and Wu 2017), and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). Bayesian inference (BI) analysis based on the dataset was performed using MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2003). The best substitution model for the dataset was selected by ModelFinder (Kalyaanamoorthy et al. 2017) using a Bayesian information criterion, and the model was used for Bayesian analysis. Four Markov chains were run from random starting trees. Trees were sampled every 1,000th generation. The first 25% of sampled trees were discarded as burn-in, whereas other trees were used to construct a 50% majority consensus tree and for calculating Bayesian posterior probabilities (BPPs). The bootstrap support for ML is greater than or equal to 70%, and Bayesian posterior probabilities greater than or equal to 0.95 are indicated on the branches in the phylogenetic tree, respectively.

Table 1. Names, voucher numbers, references, and corresponding GenBank accession numbers of the taxa used in the phylogenetic analyses. [* Indicates type materials;—indicates sequence unavailability].

Taxa	Locality	Voucher no.	GenBank accession no.		References
			ITS	28S	
Clavaria apulica	Italy	AMB 150	MT853065	MT853066	Agnell and Papetti 2020
Clavulinopsis amoena	Australia	PBM3381	—	HQ877702	Hyde et al. 2016
Clavulinopsis aspersa	China	MHHNU10153	OQ703777	OQ703794	Yan et al. 2023
Clavulinopsis aspersa	China	MHHNU10342*	OQ703778	OQ703795	Yan et al. 2023
Clavulinopsis aurantiaca	Brazil	URM<BRA>:84212*	—	KX227749	Hyde et al. 2016
Clavulinopsis aurantiaca	Brazil	URM<BRA>:84216	KC348464	NG058946	Hyde et al. 2016
Clavulinopsis bicolor	China	MHHNU10381*	OQ703780	OQ703797	Yan et al. 2023
Clavulinopsis bispora	China	MHHNU11188	OQ703782	OQ703799	Yan et al. 2023
Clavulinopsis bispora	China	MHHNU11181*	OQ703781	OQ703798	Yan et al. 2023
Clavulinopsis corallinorosacea	Australia	PBM3380	KP257144	HQ877707	Hyde et al. 2016
Clavulinopsis corniculata	USA	TENN064106	KP257145	HQ877713	Hyde et al. 2016
Clavulinopsis erubescens	China	MHHNU10290	OQ703784	OQ703801	Yan et al. 2023
Clavulinopsis erubescens	China	MHHNU8040*	OQ703783	OQ703800	Yan et al. 2023
Clavulinopsis fusiformis	USA	PBM 2804	—	EF535273	Hyde et al. 2016
Clavulinopsis fusiformis	USA	TENN064110	—	HQ877717	Hyde et al. 2016
Clavulinopsis gracillima	Canada	MO 215748	KY706170	—	Hay et al. 2019
Clavulinopsis incarnata	China	MHHNU11331	OQ703788	OQ703805	Yan et al. 2023
Clavulinopsis incarnata	China	MHHNU11330*	OQ703787	OQ703804	Yan et al. 2023
Clavulinopsis miyabeana	China	ZP-2118	MK427059	—	Chen and Zhang 2019
Clavulinopsis sulcata	Australia	PBM3379	—	HQ877709	Hyde et al. 2016
Clavulinopsis sulcata	New Zealand	PDD78241	—	DQ284904	Dentinger and McLaughlin 2006
Clavulinopsis trigonospora	China	MHHNU9186	OQ703789	OQ703806	Yan et al. 2023
Clavulinopsis trigonospora	Italy	AMB: 18557*	NR176720	NG088120	Franchi and Marchetti 2021
Clavulinopsis tropicalis	China	MHHNU10721	OQ703792	OQ703809	Yan et al. 2023
Clavulinopsis tropicalis	China	MHHNU10722*	OQ703793	OQ703810	Yan et al. 2023
Clavulinopsis wumengshanensis	**China**	**CLZhao 29651**	**PQ408630**	**PQ408635**	**Present Study**
Clavulinopsis wumengshanensis	**China**	**CLZhao 29612***	**PQ408629**	**PQ408634**	**Present Study**
Clitocybula familia	Slovakia	BRNM 736053	JF730328	JF730323	Antonín et al. 2011
Clitocybula intervenosa	São Tomé	BAP 613 SFSU*	MH414561	MH385335	Cooper 2018
Clitocybula lacerata	Italy	AMB 18779	OM422757	OM423633	Consiglio et al. 2022
Clitocybula lacerata	Czech Republic	PRM 915404	LT854054	LT854030	Antonín et al. 2019
Clitocybula oculus	USA	PBM 1156	DQ192178	DQ151452	Matheny et al. 2006
Gerronema keralense	India	CAL 1666*	NR159832	NG064531	Latha et al. 2018
Gerronema kuruvense	India	CAL 1665*	NR159831	NG064530	Latha et al. 2018
Gerronema xanthophyllum	Czech Republic	PRM 924657	LT854023	LT854023	Antonín et al. 2019
Henningsomyces bambusae	**China**	**CLZhao 33024**	**PQ408626**	**—**	**Present study**
Henningsomyces bambusae	**China**	**CLZhao 33085**	**PQ408627**	**—**	**Present study**
Henningsomyces bambusae	**China**	**CLZhao 33088***	**PQ408628**	**—**	**Present study**
Henningsomyces candidus	France	PB338	AY571044	AY571008	Bodensteiner et al. 2004
Henningsomyces candidus	Canada	T156	AY571043	—	Bodensteiner et al. 2004
Henningsomyces hengduanensis	China	LWZ 20190807-22b	OR557251	OR527277	Liu et al. 2024
Henningsomyces hengduanensis	China	LWZ 20190807-11b*	OR557250	OR527276	Liu et al. 2024
Hydropodia subalpina	Italy	AMB 18784	OM422761	OM423638	Consiglio et al. 2022
Hydropodia subalpina	Italy	AMB 18785	OM422762	OM423639	Consiglio et al. 2022
Hydropodia subalpina	Turkey	OKA TR-K364	MN701620	MN700170	Kaygusuz et al. 2020
Leucoinocybe lenta	Italy	AMB 18837	OM422765	OM423643	Consiglio et al. 2022
Leucoinocybe taniae	Italy	AMB 18838	OM422766	OM423644	Consiglio et al. 2022
Leucoinocybe taniae	Italy	AMB 18839	OM422767	OM423645	Consiglio et al. 2022
Porotheleum albodescendens	New Zealand	PDD 96321*	OL998343	OL998382	Consiglio et al. 2022
Porotheleum domingense	Dominican Republic	JBSD 131801*	OM422768	OM423646	Consiglio et al. 2022
Porotheleum fimbriatum	France	CBS 465.50	MH856711	—	Vu et al. 2019
Porotheleum parvulum	Dominican Republic	JBSD 131802*	OM422783	OM423657	Consiglio et al. 2022

Taxa	Locality	Voucher no.	GenBank accession no.		References
			ITS	28S	
Pseudohydropus commenticius	New Zealand	PDD 86984*	OL998339	OL998379	Consiglio et al. 2022
Pseudohydropus floccipes	Czech Republic	BRNM 816173	OM422758	OM423634	Consiglio et al. 2022
Pseudohydropus parafunebris	New Zealand	PDD 87227*	JQ694112	–	Consiglio et al. 2022
Pterula echo	USA	AFTOL-ID 711	DQ494693	–	Matheny et al. 2006
Radulomyces copelandii	China	Dai 15061	KU535664	KU535672	Zhao et al. 2016
Radulotubus resupinatus	China	Cui 8383*	KU535660	KU535668	Zhao et al. 2016
Rectipilus afibulatus	UK	K(M)189533*	KT893457	–	Lucas and Dentinger 2015
Xenasma bistaminatae	**China**	**CLZhao 32542***	**PQ408631**	–	**Present study**
Xenasma bistaminatae	**China**	**CLZhao 32600**	**PQ408632**	–	**Present study**
Xenasma guttulata	**China**	**CLZhao 32193***	**PQ408633**	–	**Present study**
Xenasma praeteritum	USA	Alden Dirks:ACD0185	OM009268	–	Unpublished
Xenasma pruinosum	Japan	OTU1299	MT594801	–	Unpublished
Xenasma rimicola	Australia	N.L. Bougher NLB 1449	MT537020	–	Unpublished
Xenasma rimicola	Australia	N.L. Bougher NLB 1571	MT571671	–	Unpublished

Results

The phylogeny of *Clavulinopsis*

The dataset included ITS+LSU sequences from 27 samples representing 17 taxa. The datasets had an aligned length of 2,467 characters, of which 1,775 characters are constant, 273 are variable and parsimony-uninformative, and 419 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 1359, CI = 0.6799, HI = 0.5829, RI = 0.7129, RC = 0.4847). BI analysis yielded a similar topology to ML analysis, with an average standard deviation of split frequencies of 0.012252; trees were sampled every 1,000th generation, 0.4 million in total. The effective sample size (ESS) for Bayesian analysis across the two runs is double the average ESS (avg ESS) = 201. Branches that received bootstrap support for ML ≥ 70%, MP ≥ 50%, and BI ≥ 0.95 were considered significantly supported, respectively. The ML tree was provided (Fig. 1). The phylogenetic tree (Fig. 1) reveals that the new species *Clavulinopsis wumengshanensis* is nested into the genus *Clavulinopsis* and has a close relationship with *C. aurantiocinnabarina* (Schwein.) Corner with full support (100/100/1.00).

The phylogeny of *Henningsomyces*

The dataset included ITS sequences from 31 samples representing 23 taxa. The datasets had an aligned length of 2,375 characters, of which 1,204 characters are constant, 525 are variable and parsimony-uninformative, and 646 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 2,997, CI = 0.5706, HI = 0.4294, RI = 0.5875, RC = 0.3352). The BI analysis yielded a similar topology to the ML analysis, with an average standard deviation of split frequencies of 0.007176; trees were sampled every 1,000th generation, 0.4 million in total. And the effective sample size (ESS) for Bayesian analysis across the two runs is double the average ESS (avg ESS) = 349. Branches that received bootstrap support for ML ≥ 70%, MP ≥ 50%, and BI ≥ 0.95 were considered significantly supported, respectively. The ML tree was provided (Fig. 2). The phylogenetic tree (Fig. 2) reveals the new species *Henningsomyces bambusae* nested into the genus *Henningsomyces* and has a close relationship with *H. candidus* (Pers.) Kuntze with full support (100/100/1.00).

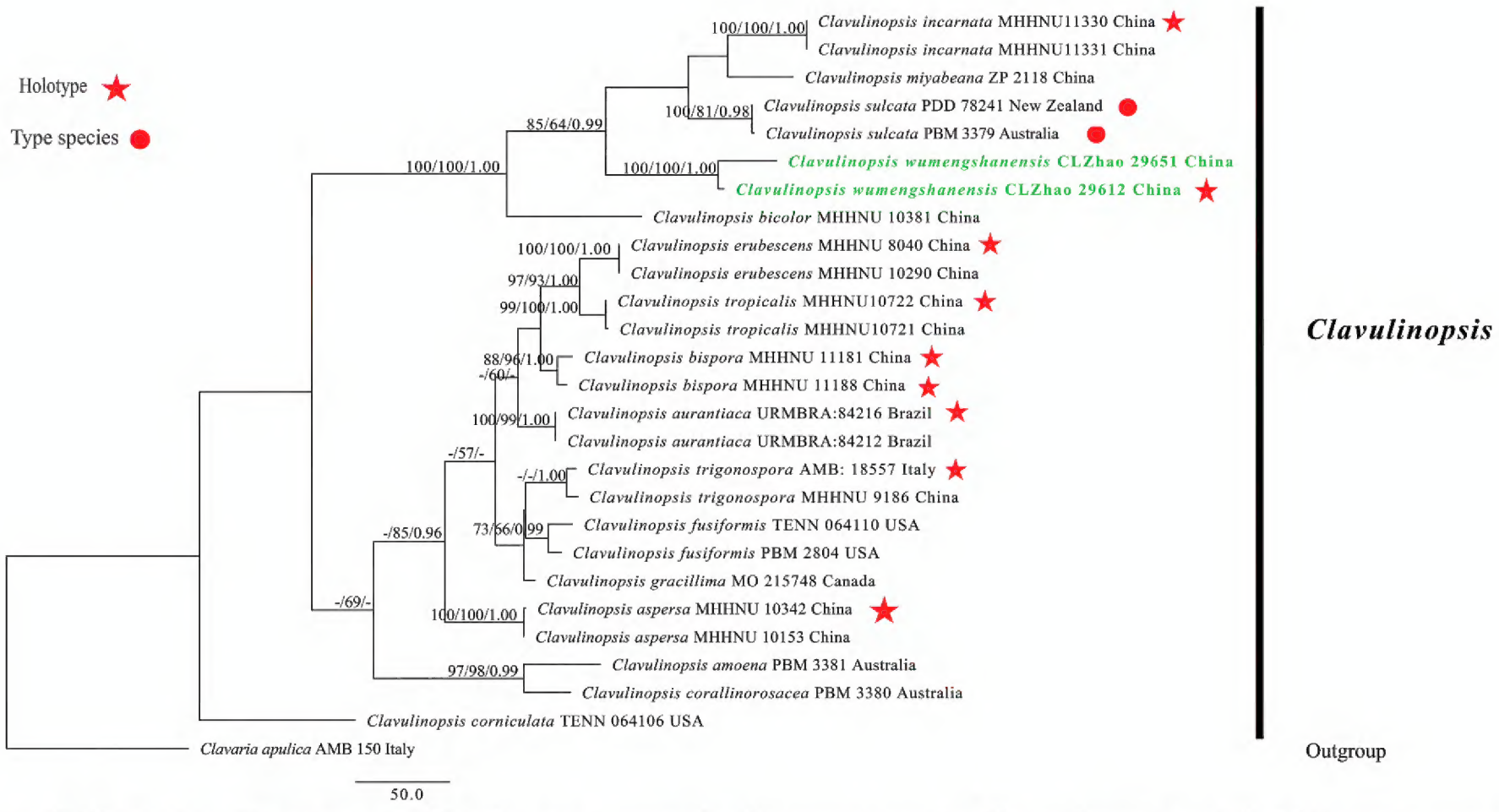

Figure 1. Phylogeny of species in *Clavulinopsis* generated by maximum likelihood based on ITS+LSU sequence data. Branches are labeled with maximum likelihood bootstrap ≥ 70%, a reduced lead value greater than 50%, and Bayesian posterior probabilities ≥ 0.95, respectively.

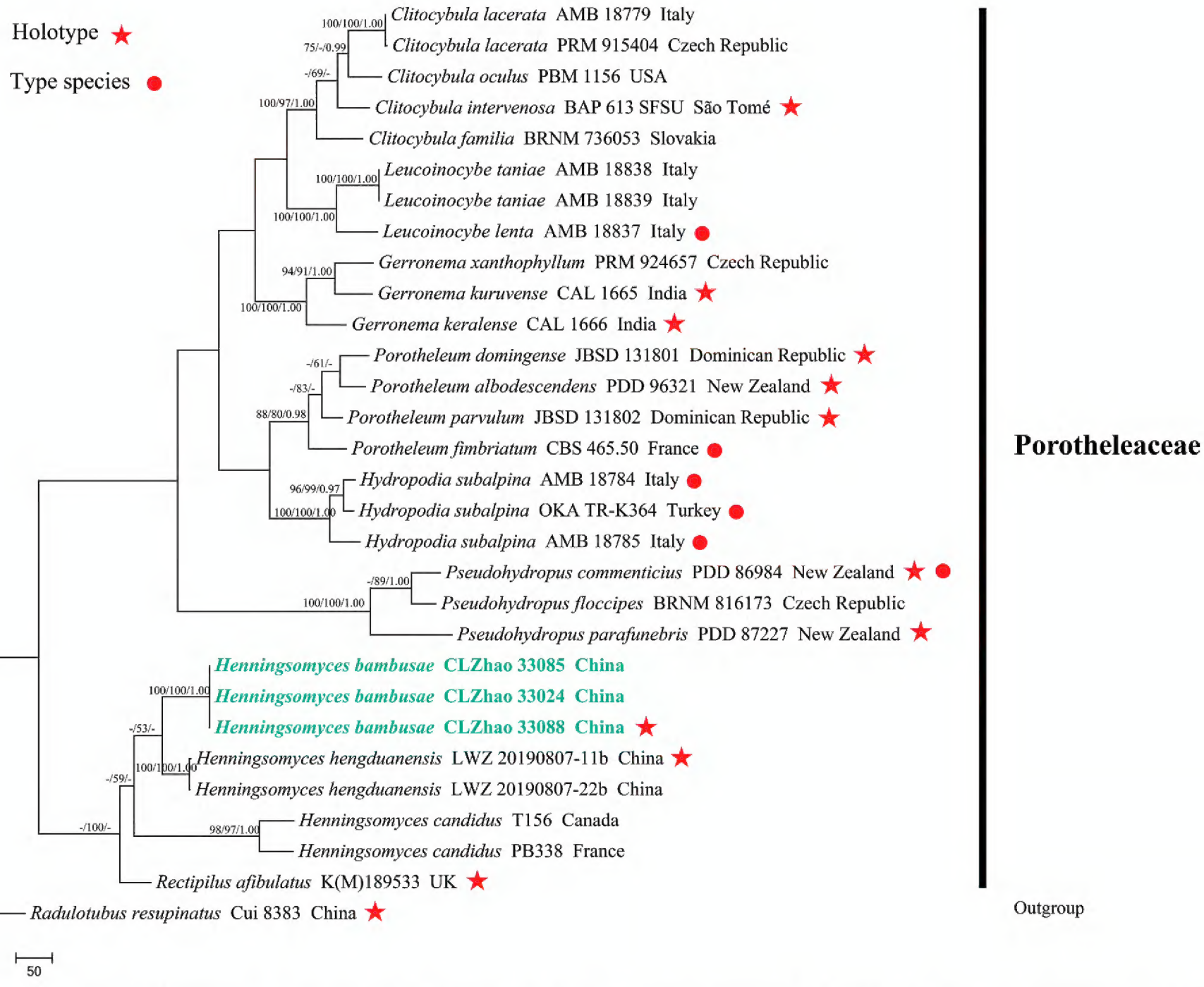

Figure 2. Phylogeny of species in *Henningsomyces* generated by maximum likelihood based on ITS sequence data. Branches are labeled with maximum likelihood bootstrap ≥ 70%, a reduced lead value greater than 50%, and Bayesian posterior probabilities ≥ 0.95, respectively.

The phylogeny of *Xenasma*

The dataset included ITS sequences from nine samples representing seven taxa. The datasets had an aligned length of 944 characters, of which 574 characters are constant, 154 are variable and parsimony-uninformative, and 216 are parsimony-informative. Maximum parsimony analysis yielded 1 equally parsimonious tree (TL = 520, CI = 0.8942, HI = 0.1058, RI = 0.8243, RC = 0.7371). The BI analysis yielded a similar topology to the ML analysis, with an average standard deviation of split frequencies of 0.007297; trees were sampled every 1,000th generation, 0.4 million in total. And the effective sample size (ESS) for Bayesian analysis across the two runs is double the average ESS (avg ESS) = 768.5. Branches that received bootstrap support for ML ≥ 70%, MP ≥ 50%, and BI ≥ 0.95 were considered significantly supported, respectively. The ML tree was provided (Fig. 3). The phylogenetic tree (Fig. 3) reveals the two new species, *Xenasma bisterigmatae* and *X. guttulate*, nested into the genus *Xenasma*. The taxon *X. bisterigmatae* has a close relationship with *X. rimicola* (P. Karst.) Donk, while the taxon *X. guttulata* has a close relationship with *X. pruinosum* with strong support (97/100/0.96).

The BLAST result of four new species for the closest top 10 taxa and their corresponding parameters are given (Table 2).

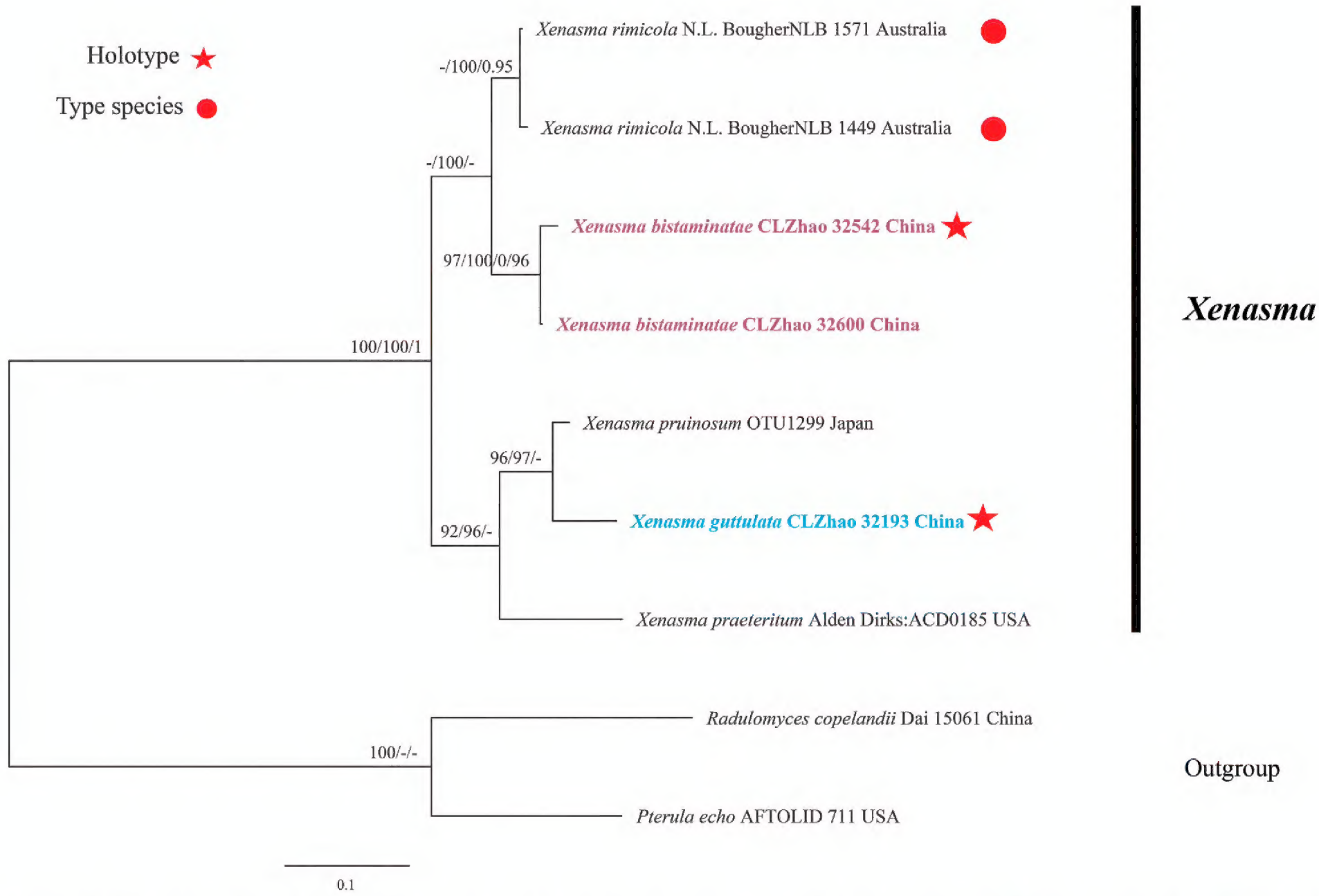

Figure 3. Phylogeny of species in *Xenasma* generated by maximum likelihood based on ITS sequence data. Branches are labeled with maximum likelihood bootstrap ≥ 70%, a reduced lead value greater than 50%, and Bayesian posterior probabilities ≥ 0.95, respectively.

Table 2. The BLAST result of four new species for the closest top 10 taxa and their corresponding parameters.

Scientific Name	Specimens	Sequence Number	Max score	Total score	Query cover	E value	Per. Ident.	Acc. len.	Accession
Clavulinopsis wumengshanensis	CLZhao 29612	PQ408629	1123	1123	100%	0	100%	638	PQ408629
			734	734	71%	0	97.24%	431	PQ515870
			448	448	44%	6e-121	96.68%	295	PQ453614
			911	911	93%	0	95.94%	573	ON943319
			972	972	100%	0	95.60%	627	PQ346233
			970	970	100%	0	95.59%	626	PQ346239
			976	976	100%	0	95.57%	624	PQ346237
			966	966	100%	0	95.43%	653	PQ453611
			966	966	100%	0	95.43%	632	PQ346229
			966	966	100%	0	95.43%	627	PQ346265
Clavulinopsis wumengshanensis	CLZhao 29612	PQ408634	1024	1024	100%	0	100.00%	638	PQ408629
			896	896	100%	0	95.85%	643	PQ408630
			885	885	99%	0	95.99%	627	PQ346233
			881	881	100%	0	95.32%	624	PQ346237
			880	880	99%	0	95.81%	611	PQ453612
			880	880	99%	0	95.81%	653	PQ453611
			880	880	99%	0	95.81%	626	PQ346239
			880	880	99%	0	95.81%	632	PQ346229
			880	880	99%	0	95.81%	627	PQ346265
			878	878	99%	0	95.63%	590	PQ453613
Henningsomyces bambusae	CLZhao 33088	PQ408628	1282	1282	100%	0	100.00%	694	PQ408628
			1253	1253	98%	0	99.85%	694	PQ408626
			1223	1223	97%	0	99.26%	697	PQ408627
			1081	1081	96%	0	95.71%	701	LC774058
			1077	1077	96%	0	95.56%	733	PP849903
			1072	1072	96%	0	95.41%	732	MG707601
			1059	1059	96%	0	951%	745	MK607599
			985	985	89%	0	95.22%	643	AB847016
			950	950	89%	0	94.38%	622	AY571057
			813	813	90%	0	903%	652	OQ872100
Xenasma bisterigmatae	CLZhao 32542	PQ408631	1138	1138	100%	0	100.00%	616	PQ408631
			1040	1040	99%	0	97.55%	611	PQ408632
			846	846	92%	0	93.58%	634	MT537020
			758	676	83%	0	92.83%	676	MT571671
			597	597	60%	6e-166	95.48%	411	LR819650
			597	597	60%	6e-166	95.48%	411	LR602855
			508	508	72%	3e-139	87.25%	636	JF691144
			503	503	91%	1e-137	86.96%	675	PQ408633
			492	492	59%	3e-134	90.98%	638	OM009268
			431	431	60%	6e-116	87.47%	417	LR819360
Xenasma guttulata	CLZhao 32193	PQ408633	1247	1247	100%	0	100.00%	675	PQ408633
			737	737	91%	0	88.36%	636	JF691144
			634	634	91%	5e-177	85.53%	638	OM009268
			592	592	55%	3e-164	94.97%	417	LR819360
			592	592	55%	3e-164	94.97%	417	LR602599
			586	586	55%	1e-162	94.71%	417	LR602541
			586	586	55%	1e-162	94.71%	417	LR819292
			558	558	55%	3e-154	93.39%	417	LR602585
			558	558	55%	3e-154	93.39%	417	LR819344
			507	507	47%	1e-138	95.34%	417	MT852420

Taxonomy

Clavulinopsis wumengshanensis **S.Y. He., H.M. Zhou & C.L. Zhao, sp. nov.**
MycoBank No: 855906
Figs 4, 5

Diagnosis. *Clavulinopsis wumengshanensis* differs from *C. aurantiocinnabarina* by buff-yellow to straw-yellow basidiomata and thick-walled, subglobose, and longer basidiospores (7–8.5 µm vs. 5.6–7.1 µm).

Figure 4. Basidiomata of *Clavulinopsis wumengshanensis* (holotype, CLZhao 29612). Scale bars: 1 cm (**A**); 1 mm (**B**).

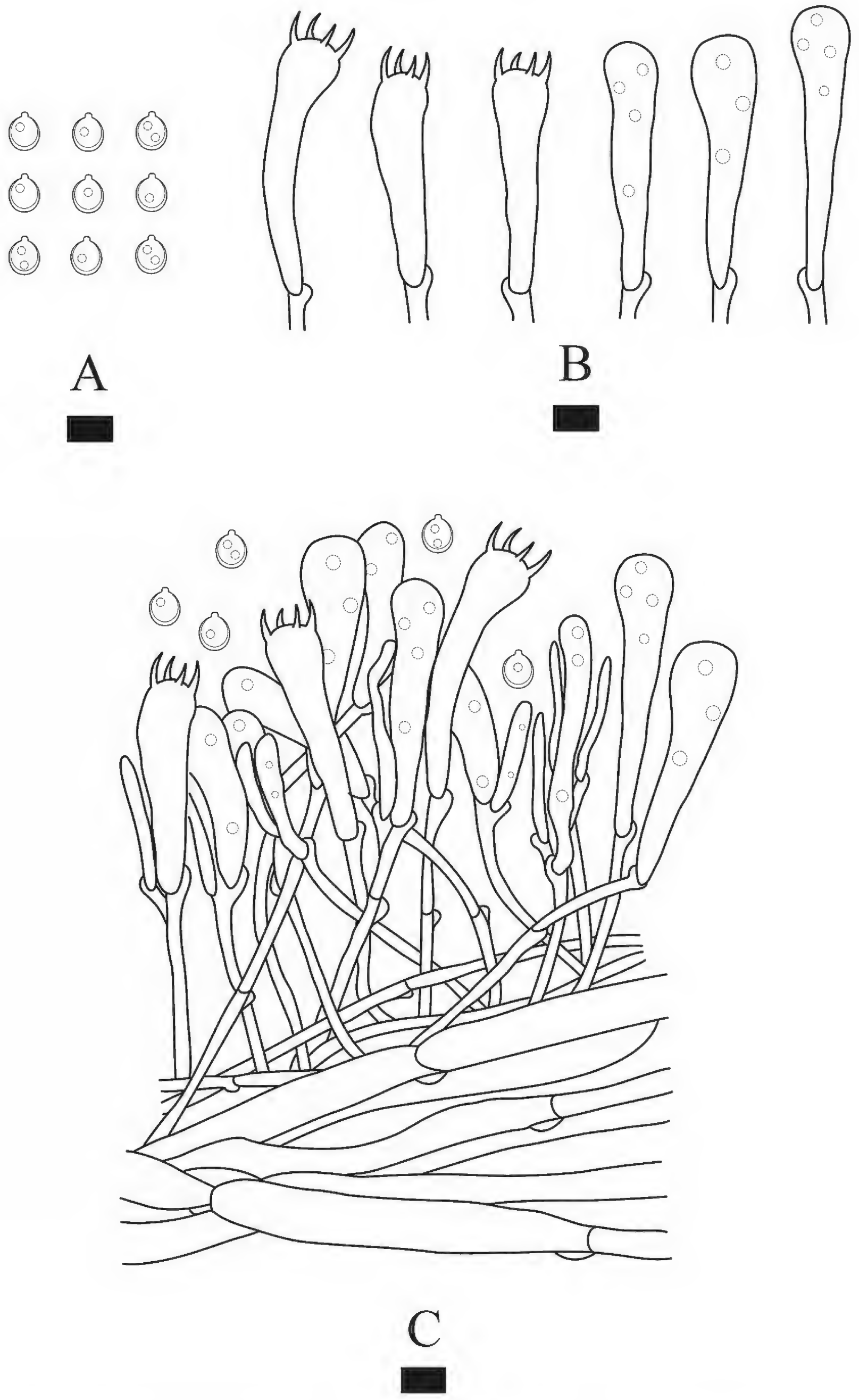

Figure 5. Microscopic structures of *Clavulinopsis wumengshanensis* (holotype, CLZhao 29612) **A** basidiospores **B** basidia and basidioles **C** a section of basidiomata. Scale bars: 10 μm (**A–C**).

Holotype. CHINA • Yunnan Province, Zhaotong, Yiliang County, Wumengshan National Nature Reserve, GPS coordinates 27°30'N, 104°12'E, evel. 1710 m asl., on the ground, leg. C.L. Zhao, 12 July 2023, CLZhao 29612 (SWFC).

Etymology. *Wumengshanensis* (Lat.) refers to the locality "Wumengshan National Nature Reserve" of the holotype.

Basidiomata. Basidiomes annual, clavarioid, without odor or taste when fresh, up to 8 cm long, 4 mm wide, and 400–800 μm thick. Fertile part subcylindrical to fusiform, occasionally slightly curved or flexuous and with a distinct longitudinal depression, buff-yellow (4A4) to straw-yellow (3A/B3) when dry. Apex rounded or obtuse acute when mature, concolourous or slightly paler.

Hyphal structure. Monomitic, generative hyphae with clamp connections, hyaline, thick-walled, parallel, interwoven, 2–3 μm in diam, some inflated to 13 μm in diam, IKI–, CB–; tissues unchanged in KOH. Cystidia absent. Basidia clavate to subcylindrical, hyaline, thin-walled, with four sterigmata and a basal clamp connection, 35–48.5 × 9–11.5 μm; basidioles in shape similar to basidia, with several guttules.

Spores. Basidiospores subglobose with a distinct apiculus, hyaline, thick-walled, smooth, with several guttules, IKI–, CB–, 7–8.5(–5) × (5.5–)6–7.5 μm, L = 7.59 μm, W = 6.54 μm, Q = 1.14–1.16 (n = 60/2).

Additional specimen examined (*paratype*). CHINA • Yunnan Province, Zhaotong, Yiliang County, Wumengshan National Nature Reserve, GPS coordinates 27°30'N, 104°12'E, evel. 1710 m asl., on the ground, leg. C.L. Zhao, 12 July 2023, CLZhao 29651 (SWFC).

Notes. Based on the ITS + LSU analysis (Fig. 1), the result showed that the new species *Clavulinopsis wumengshanensis* is grouped with *C. aurantiocinnabarina* (Schwein.) Corner. However, *C. aurantiocinnabarina* differs from *C. wumengshanensis* by its thin-walled basidiospores and narrower basidia (5.2–7.1 μm vs. 9–11.5 μm, Petersen 1978).

***Henningsomyces bambusae* S.Y. He, H.M. Zhou & C.L. Zhao, sp. nov.**

MycoBank No: 855907

Figs 6, 7

Diagnosis. *Henningsomyces bambusae* differs from *H. candidus* by its globose to subglobose and wider basidiospores (6.5–8.5 μm vs. 4–5 μm).

Holotype. CHINA • Yunnan Province, Zhaotong, Wumengshan National Nature Reserve, GPS coordinates 27°29'N, 103°55'E, evel. 1900 m asl., on dead bamboo, leg. C.L. Zhao, 18 September 2023, CLZhao 33088 (SWFC).

Etymology. *Bambusae* (Lat.) refers to the host genus *Bambusa*.

Basidiomata. Basidiomes forming loosely scattered colonies, short cylindric to tubular, sessile, up to 1–1.6 cm in length, 0.5–1 cm diam; external surface white (60) to cream (4A2/3), finely tomentose when dry; inner surface covered by a deeply concave, smooth hymenium.

Hyphal structure. Monomitic, generative hyphae with clamp connections and simple septa, thin- to thick-walled, IKI–, CB–; tissues unchanged in KOH. External hyphae slightly contorted, widest at the base, slightly tapering towards the obtusely rounded apex, hyaline, thick-walled to solid, occasionally with

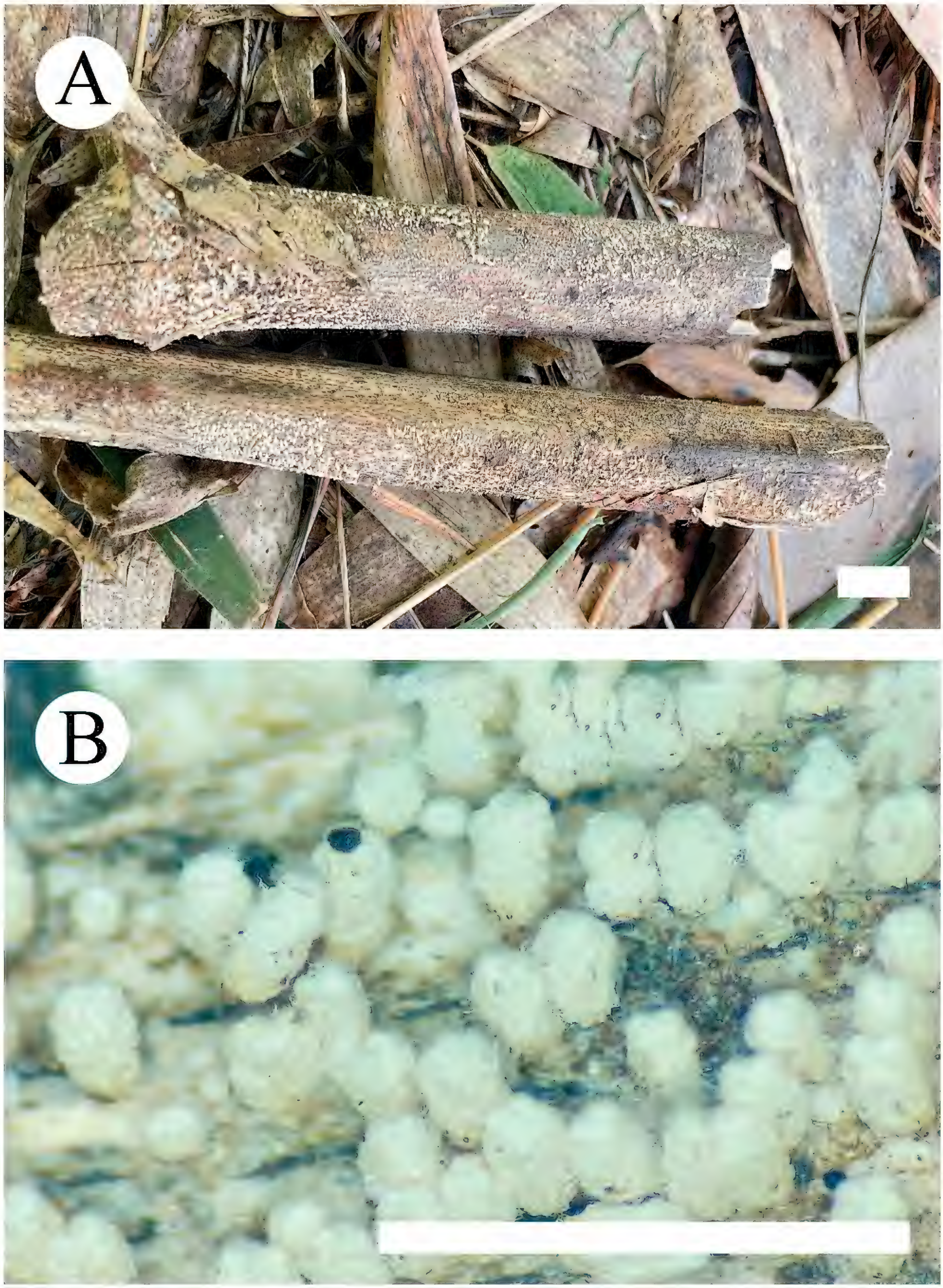

Figure 6. Basidiomata of *Henningsomyces bambusae* (holotype, CLZhao 33088). Scale bars: 1 cm (**A**); 1 mm (**B**).

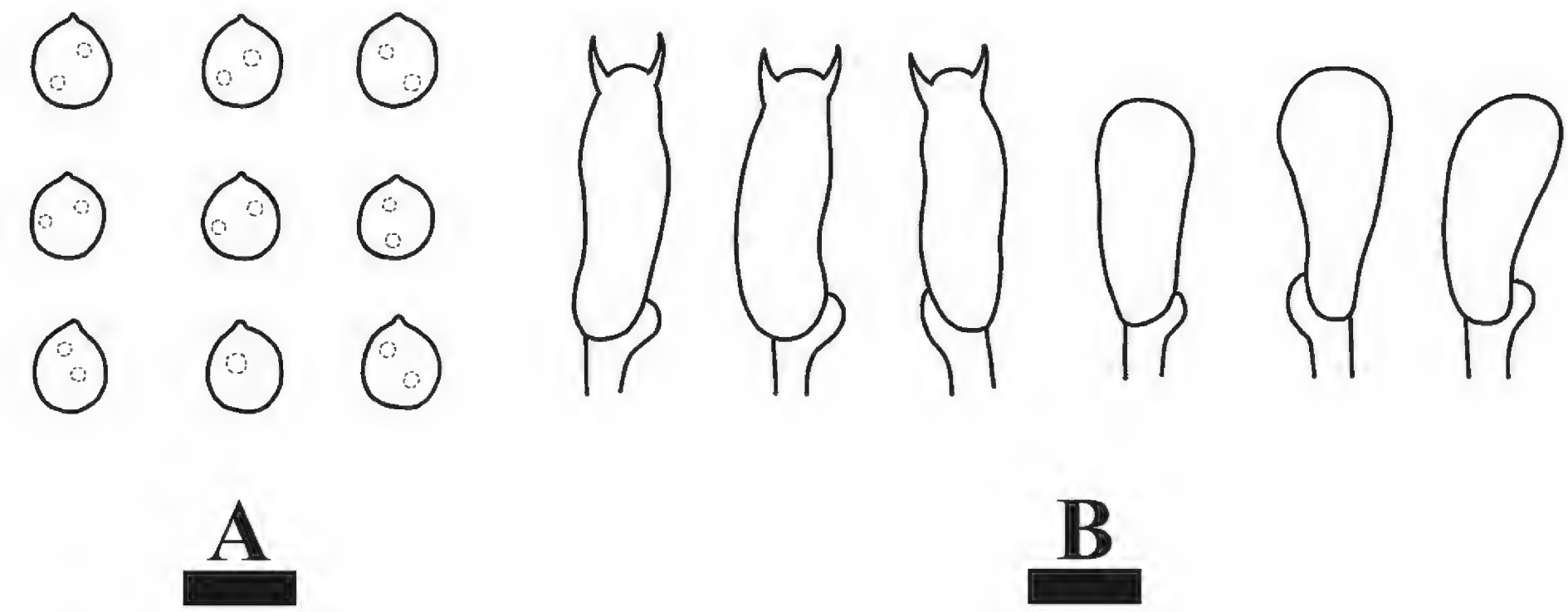

Figure 7. Microscopic structures of *Henningsomyces bambusae* (holotype, CLZhao 33088) **A** basidiospores **B** basidia and basidioles **C** a section of basidiomata. Scale bars: 10 μm (**A–C**).

simple septa, frequently unbranched, dextrinoid, CB−, 3–4 μm diam; tramal hyphae hyaline, thin-walled, with clamp connections, IKI−, 2.5–3 μm diam. Cystidia absent. Basidia cylindrical to subcylindrical, hyaline, thin-walled, with two

sterigmata and a basal clamp connection, 19.5–22.5 × 7–8.5 µm; basidioles in shape similar to basidia, but slightly smaller.

Spores. Basidiospores globose to subglobose, hyaline, thin-walled, smooth, with guttules, IKI–, CB–, 6.5–8.5(–9) × (6–)6.5–8.5(–9) µm, Lm = 7.61 µm, Wm = 7.45 µm, Q = 1.02–1.04 (n = 60/2).

Additional specimens examined (*paratypes*). CHINA • Yunnan Province, Zhaotong, Wumengshan National Nature Reserve, GPS coordinates 27°29'N, 103°55'E, evel. 1900 m asl., on a dead bamboo, leg. C.L. Zhao, 18 September 2023, CLZhao 33024; CLZhao 33085 (SWFC).

Notes. Based on the ITS analysis (Fig. 2), the new taxon *Henningsomyces bambusae* grouped within the genus *Henningsomyces* and formed a sister group with *H. candidus* (Pers.) Kuntze. However, *H. candidus* can be delimited from *H. bambusae* by its narrower basidiospores (4–5 µm vs. 6–8 µm; Gilbertson and Blackwell 1985).

Xenasma bisterigmatae **S.Y. He, H.M. Zhou & C.L. Zhao, sp. nov.**

MycoBank No: 855908

Figs 8, 9

Diagnosis. *Xenasma bisterigmatae* differs from *X. rimicola* by its ellipsoid to subglobose and larger basidiospores (8.5–10.5 × 4.5–7 µm vs. 10–12.5 × 8–10.5 µm), two-sterigmata basidia.

Holotype. CHINA • Yunnan Province, Zhaotong, Wumengshan National Nature Reserve, GPS coordinates 27°29'N, 103°55'E, evel. 1900 m asl., on fallen angiosperm branch, leg. C.L. Zhao, 29 August 2023, CLZhao 32542 (SWFC).

Etymology. *Bisterigmatae* (Lat.) refers to two-sterigmata basidia of the holotype.

Basidiomata. Basidiomes annual, resupinate, smooth, without odor or taste when fresh, up to 6.5 cm long, 2.3 cm wide, and 100 µm thick. Hymenial surface smooth, membranaceous, ash-grey (19C2) when dry; sterile margin indistinct.

Hyphal structure. Monomitic, generative hyphae with clamp connections, thin-walled, branched, interwoven, with dense crystal, 3.2–3.5 µm diam, IKI–, CB–; tissues unchanged in KOH. Cystidia abundant, tubular with obtuse apex, with slightly thick walls in the basal part that appears frequently collapsed, often with an apical amorphous globule, 64.5–93 × 5.5–7.5 µm. Cystidioles absent. Basidia clavate to subcylindrical, thin-walled, with two sterigmata and a basal clamp connection, with several guttules, 21.5–28 × 10–12 µm; basidioles in shape similar to basidia, but slightly smaller.

Spores. Basidiospores ellipsoid to subglobose, thick-walled, verrucose, IKI–, CB–, 10–12.5 × 8–10.5(–11) µm, Lm = 11.15 µm, Wm = 9.3 µm, Q = 1.19–1.27 (n = 60/2).

Additional specimens examined (*paratype*). CHINA • Yunnan Province, Zhaotong, Wumengshan National Nature Reserve, GPS coordinates 27°29'N, 103°55'E, evel. 1900 m asl., on fallen angiosperm branch, leg. C.L. Zhao, 29 August 2023, CLZhao 32600 (SWFC).

Notes. Based on the ITS analysis (Fig. 3), the new species *Xenasma bisterigmatae* was clustered with *X. rimicola* (P. Karst.) Donk. However, *X. rimicola* differs from *X. bisterigmatae* by its four-sterigmata basidia (Cunningham 1963).

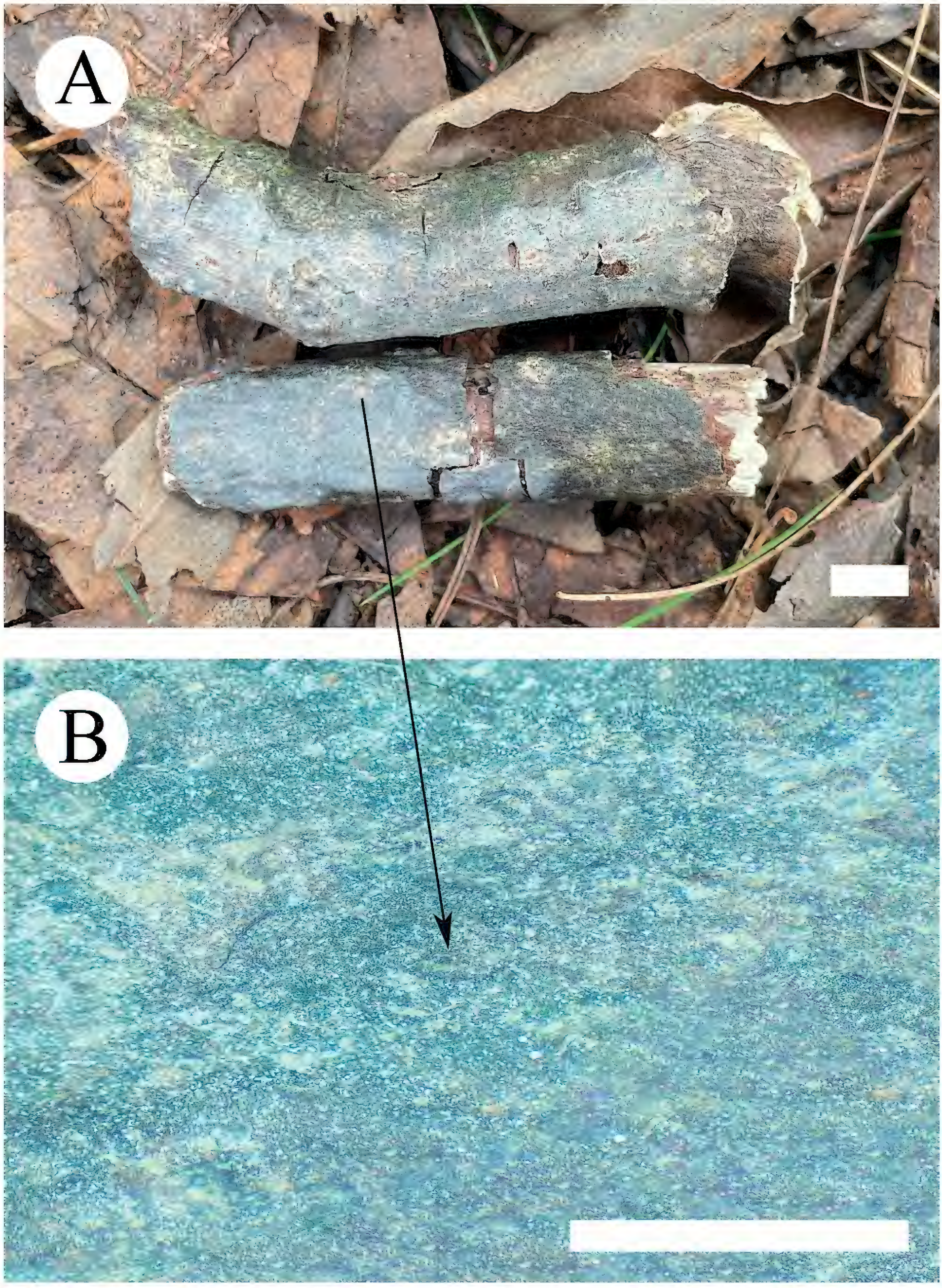

Figure 8. Basidiomata of *Xenasma bisterigmatae* (holotype, CLZhao 32542). Scale bars: 1 cm (**A**); 1 mm (**B**).

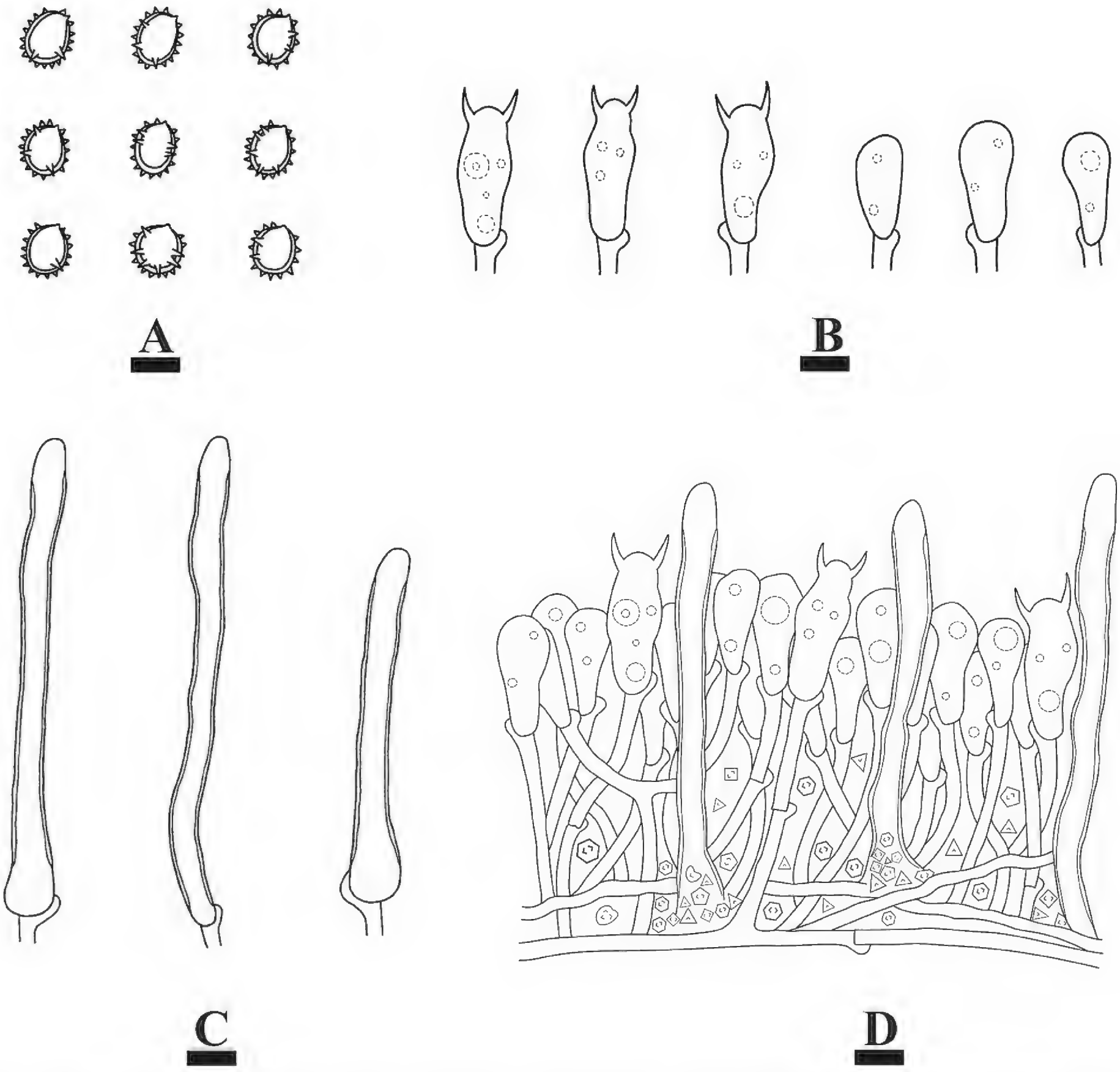

Figure 9. Microscopic structures of *Xenasma bisterigmatae* (holotype, CLZhao 32542) **A** basidiospores **B** basidia and basidioles **C** cystidia **D** a section of basidiomata. Scale bars: 10 μm (**A–C**).

Xenasma guttulata **S.Y. He, H.M. Zhou & C.L. Zhao, sp. nov.**

MycoBank No: 855909

Figs 10, 11

Diagnosis. *Xenasma guttulata* differs from *X. pruinosum* by its membranaceous basidiomata with a white to cream hymenial surface and larger basidiospores (6–7 × 3–4 μm vs. 7–9 × 5.5–7.5 μm).

Holotype. China • Yunnan Province, Zhaotong, Wumengshan National Nature Reserve, GPS coordinates 27°29'N, 103°55'E, evel. 1900 m asl., on a fallen angiosperm branch, leg. C.L. Zhao, 29 August 2023, CLZhao 32193 (SWFC).

Etymology. *Guttulata* (Lat.) refers to the basidiospores with guttules in the holotype.

Basidiomata. Basidiomes annual, resupinate, closely adnate, without odor or taste when fresh, up to 2.5 cm long, 1.5 cm wide, and 100 μm thick. Hymenial

surface smooth, membranaceous, white (60) to cream (4A2/3) when dry; sterile margin indistinct.

Hyphal structure. Monomitic, generative hyphae with clamp connections, thin-walled, occasionally branched, interwoven, with dense crystal, 3.3–3.5 µm diam, IKI–, CB–; tissues unchanged in KOH. Cystidia abundant, cystidia tubular with obtuse apex, 75–91.5 × 4–8.5 µm, with slightly thick walls in the basal part that appears frequently collapsed, often with an apical amorphous globule.

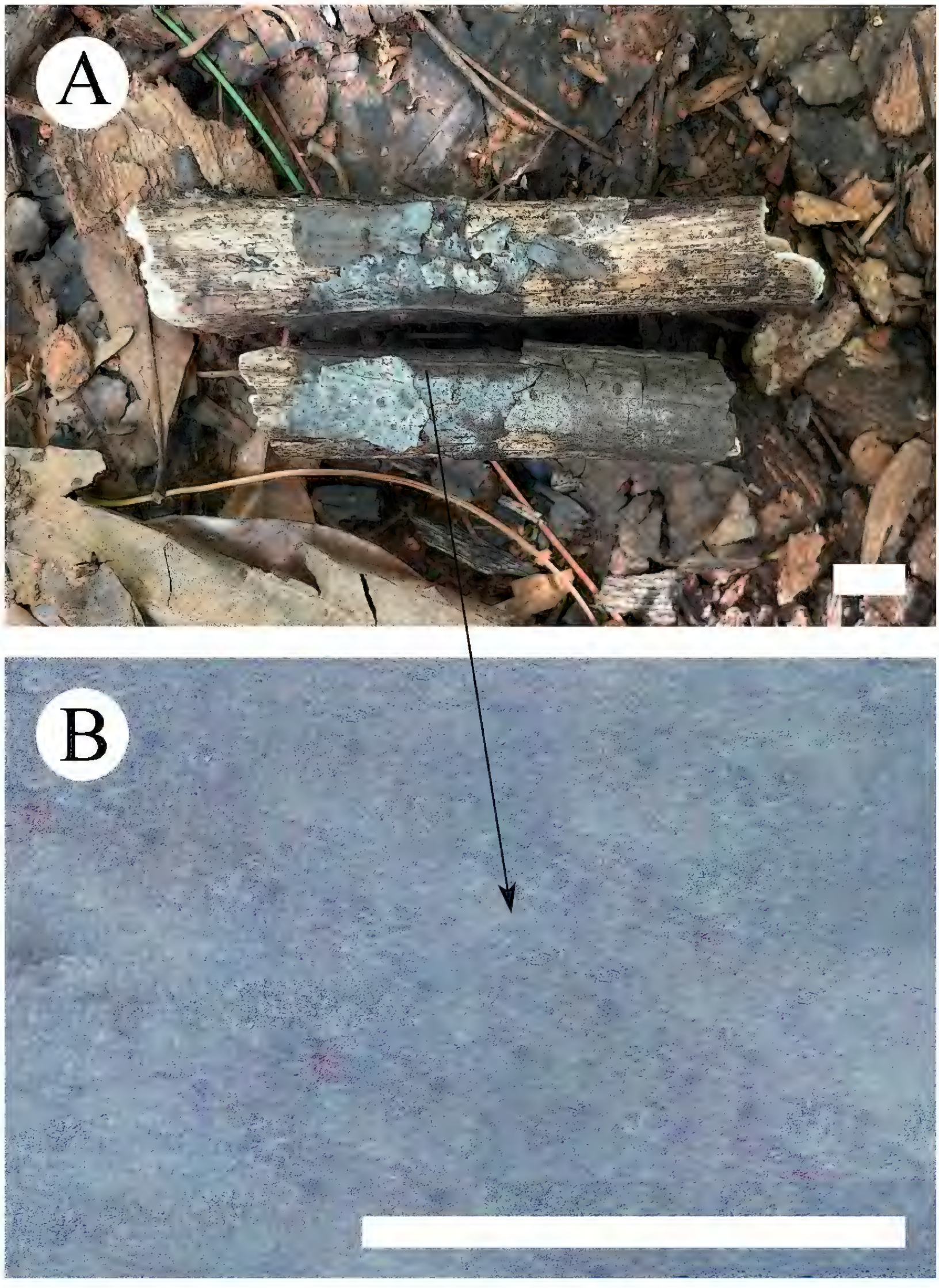

Figure 10. Basidiomata of *Xenasma guttulata* (holotype, CLZhao 32193). Scale bars: 1 cm (**A**); 1 mm (**B**).

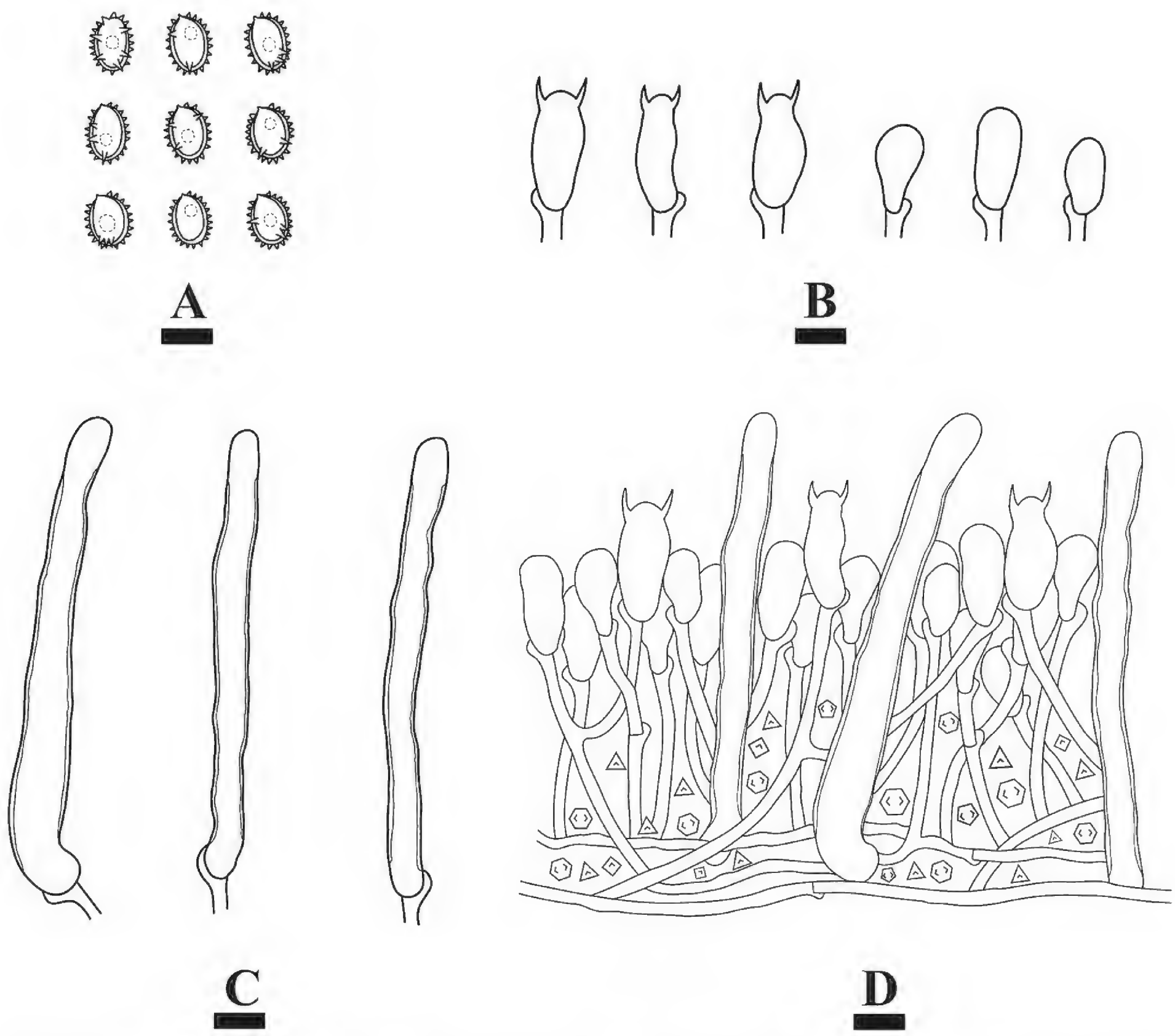

Figure 11. Microscopic structures of *Xenasma guttulata* (holotype, CLZhao 32193) **A** basidiospores **B** basidia and basidioles **C** cystidia **D** a section of basidiomata. Scale bars: 10 μm (**A–C**).

Cystidioles absent. Basidia clavate to subcylindrical, thin-walled, with two sterigmata and a basal clamp connection, 18.5–24 × 7.5–11 μm; basidioles in shape similar to basidia, but slightly smaller.

Spores. Basidiospores ellipsoid to narrowly ellipsoid, thick-walled, verrucose, with several guttules, IKI–, CB–, 7–9 × 5.5–7.5 μm, Lm = 8.05 μm, Wm = 6.52 μm, Q = 1.23 (n = 30/1).

Notes. Based on the ITS analysis (Fig. 3), the new species *Xenasma guttulata* was clustered with *X. pruinosum* (Pat.) Donk. However, *X. pruinosum* can be distinguished from *X. guttulata* by its smaller basidiospores (6–7 × 3–4 μm vs. 7–9 × 5.5–7.5; Donk 1957; Bernicchia and Gorjón 2010).

Discussion

The multilocus (ITS+LSU) analysis (Fig. 1) showed that the new species *Clavulinopsis wumengshanensis* groups with *C. aurantiocinnabarina* (Schwein.) Corner. However, *C. aurantiocinnabarina* differs from *C. wumengshanensis* by its

thin-walled basidiospores and narrower basidia (5.2–7.1 µm vs. 9–11.5 µm) (Petersen 1978). Morphologically, *Clavulinopsis fusiformis* (Sowerby) Corner and *C. incarnata* P. Zhang & Jun Yan are similar to *C. wumengshanensis* by both having smooth, globose to subglobose basidiospores (Keleş and Kaya 2021; Yan et al. 2023). However, *C. fusiformis* differs in its pale yellow basidiomata, and *C. incarnata* differs in its pinkish basidiomata (Keleş and Kaya 2021; Yan et al. 2023).

Based on the ITS locus phylogenetic analysis (Fig. 2), the new taxon *Henningsomyces bambusae* forms a sister group with *H. candidus* (Pers.) Kuntze. However, *H. candidus* can be delimited from *H. bambusae* by its narrower basidiospores (4–5 µm vs. 6–8 µm, Gilbertson and Blackwell 1985). Morphologically, *Henningsomyces hengduanensis* S.L. Liu & L.W. Zhou and *H. minimus* (Cooke and W. Phillips) Kuntze are similar to *H. bambusae* by both having thin-walled, smooth, subglobose basidiospores (Wei and Qin 2009; Liu et al. 2023). However, *H. hengduanensis* differs in its cream basidiomata and broadly clavate basidia with four sterigmata (Liu et al. 2023); *Henningsomyces minimus* differs in its shorter (11–15 µm vs. 19.5–22.5 µm), broadly clavate basidia with four sterigmata (Wei and Qin 2009).

Based on the combined ITS locus phylogeny (Fig. 3), *X. rimicola* (P. Karst.) Donk differs from the new species *X. bisterigmatae* by its four-sterigmata basida (Cunningham 1963); *X. pruinosum* (Pat.) Donk can be distinguished from the new species *X. guttulata* by its smaller basidiospores (6–7 × 3–4 µm vs. 7–9 × 5.5–7.5, Donk 1957; Bernicchia and Gorjón 2010). Morphologically, *X. pruinosum* is similar to *X. bisterigmatae* by having tubular cystidia with an apical amorphous globule and slightly thick walls in the basal part (Donk 1957; Bernicchia and Gorjón 2010). However, *X. pruinosum* differs in its smaller basidiospores (6–7 × 3–4 µm vs. 7–9 × 5.5–7.5 µm; Donk 1957; Bernicchia and Gorjón 2010). *Xenasma rimicola* is similar to *X. guttulata* by having tubular cystidia and slightly thick walls in the basal part (Donk 1957). However, *X. rimicola* differs in its subclavate basidia with four sterigmata (Donk 1957).

Yunnan Province is located in the southwest of China. The climate in Yunnan offers conducive environments for the speciation and diversification of various life forms, and this climatic diversity in Yunnan creates varied landscapes with multiple habitats, resulting in a high species diversity, with over 6,000 recorded fungal species up to now (Wang and Cai 2022; Dong et al. 2024; Wang et al. 2024; Zhou et al. 2024b). Therefore, focusing on the diversity of fungi in the Yunnan-Guizhou Plateau of China is of great significance. Based on the present study, the results not only enrich the species diversity of fungi worldwide but also contribute to the branches of the fungal tree of life.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Data curation: HSY, WL, ZHM. Formal analysis: HSY, WL, SKZ, ZHM. Methodology: HSY, ZHM. Software: HSY, ZHM. Writing—original draft: HSY, WL, SKZ. Writing—review and editing: ZHM.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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